

Response surface optimization of the ultrasonic-assisted extraction of edible brown pigment from Macadamia shells

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Abstract. The ultrasonic extraction of Edible brown pigment from macadamia shells was researched using response surface methodology (RSM) with 3 factors and 3 levels. A Box-Behnken design (BBD) was employed to investigate the effects of Solvent concentration, ratio of water to raw material and extraction time on the extraction yield of brown pigment. By using this new method, the optimum extraction condition was obtained as follows: Ultrasonic treating time 71 min, solvent to sample ratio of 23 mL/g, Alcohol concentrations 62%. Under the optimized condition, the experimental yield of brown pigment was 0.636g.

1 Introduction

Edible pigments which are able to divide into synthetic pigment and natural pigments, as an important branch of food additives, are widely applied to the food, cosmetic, pharmaceutical and other industries. Synthetic pigments are good stability, and low cost. Natural pigments are mainly extracted from plants, which are of high safety [5]. On the one hand, with the development of medical toxicology and biological test work, some serious chronic diseases and cancers caused by chemical synthetic pigment are continued to be found, on the other hand, natural pigments are famous of its high safety, some of which are able to prevent and treat many of diseases. Increasingly, returning to nature becomes a main stream [8].

Macadamia was introduced into Guangdong Province, China, as an economic crop in the late 1970s, and now the planting area are extended to Guangxi, Yunnan, and Fujian Provinces. With the planting area and productive gradually increasing, avoiding the pollution caused by macadamia shell was attracting us attention [3]. The macadamia shells contain many nature active components, such as flavones. Making the extractions from macadamia shells into a food additive can prompt nutritive value, while decreasing the pollution.

Response surface methodology has been successfully used to model and optimize biochemical and biotechnological processes related to food systems [1,4,7] including extraction of phenolic compounds from berries and vitamin E from wheat germ. The extraction and purification of phytochemicals from natural sources is needed, since these bioactives are often used in the preparation of dietary supplements, nutraceuticals, functional food ingredients, and food additives, pharmaceutical and cosmetic products [2,6]. In this study, optimization of experimental conditions that results in the most weight of edible brown pigment was conducted. The target is that a kind of natural pigment from the shells can be gained as much as possible.



2 Materials and Methods

2.1 Materials and chemicals

Macadamia from Yunnan Province, China. The agents such as ethanol are purchased from Sigma. The distilled water was used in the experiment.

2.2 Extraction of Brown Pigment

Air-dried Macadamia shells were ground in a high speed disintegrator to obtain a fine powder, and 15g the powder through a 60 mesh sieve was extracted ultrasonically with ethanol solution (from 0% to 100%). After separating the liquid from mixer, the brown pigment can be obtain by evaporating the ethanol solvent.

2.3 Design of statistical experiments

A three-level-factor, BBD was employed in optimizing the extraction conditions for high recovery of brown pigment from Macadamia shells, after determining the preliminary range of extraction variables through single-factor test. Three independent variables namely solvent concentration (X_1), solvent to sample ratio (X_2), extraction time (X_3) were coded at three levels, -1, 0, and +1 for high, intermediate and low value respectively. The range of independent variables and their levels were presented in Table 1.

Table 1: Independent variables and their levels used in the response surface design

Independent variables	Levels		
	-1	0	1
Solvent concentration	55	60	65
solvent to sample ratio	17:1	20:1	23:1
extraction time	65	70	75

The statistical analysis of the model was performed in the form of analysis of variance. Regression analysis was performed and fitted into the empirical second-order polynomial model for the experimental data as shown in equation:

$$Y = A_0 + \sum_{i=1}^3 A_i X_i + \sum_{i=1}^3 A_{ii} X_i^2 + \sum_{i < j=1}^3 \sum_{j=1}^3 A_{ij} X_i X_j$$

Where A_0 , A_i , A_{ii} , and A_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i , and X_j are the independent variables, and Y is the dependent variable (the quality of brown pigment). The designed variables in coded units are given in Table 2 along with experimental values.

Table 2 Box-Behnken experimental design and results for extraction yield

NO.	X1 (solvent concentration)	X2 (solvent to sample ratio)	X3 (extraction time)	Yield
1	1	0	-1	0.5788
2	-1	-1	0	0.4908
3	1	0	1	0.5836
4	0	0	0	0.6033
5	0	-1	-1	0.5027
6	-1	0	1	0.5605
7	-1	0	-1	0.5385
8	0	1	-1	0.6071
9	0	1	1	0.6174
10	0	0	0	0.6004
11	1	1	0	0.6316

12	-1	1	0	0.6086
13	0	0	0	0.5983
14	0	-1	1	0.4962
15	1	-1	0	0.5504
16	0	0	0	0.6035
17	0	0	0	0.6012

3 Results and Discussions

3.1 Response surface models fitting

The effects of three main variables on brown pigment from Macadamia shells were simultaneously investigated using a three-factor design with three levels for each factor (low, medium, and high). The main aim of the optimization process was to maximize the brown pigment concentrations in ethanol extraction with ultrasonic-assisted.

The data obtained in the Box-Behnken experiment were converted into second-order polynomial equation with three independent variables and responses.

$$Y = +0.60 + 0.018X_1 + 0.053X_2 + 3.825 \times 10^{-3}X_3 - 9.150 \times 10^{-3}X_1X_2 - 4.300 \times 10^{-3}X_1X_3 + 4.200 \times 10^{-3}X_2X_3 - 0.011X_1^2 - 0.020X_2^2 - 0.025X_3^2$$

Where Y is the yield of brown pigment (g), and X_1 , X_2 , X_3 are the coded variables for alcohol concentrations, solvent: sample ratio, extraction time, respectively.

Table 3 listed the analysis of variance for the fitted quadratic polynomial model of extraction yield of brown pigments, and the significance of each coefficient was determined by F-test and P-value. The Model F-value of 110.62 implies the model is significant. Values of “Prob > F” are less than 0.0500 indicate that model terms are significant. It can be seen from Table 3 that the independent variables (X_1, X_2), interaction term (X_1X_2) and quadratic terms (X_1^2, X_2^2, X_3^2) exhibit significant effects on the yield of brown pigment, meanwhile, X_1, X_2 and X_3 were the major factor affecting the yield of brown pigment. The lack of fit measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression. As showed in table 3 F-value and P-value of the lack of fit were 14.34 and 0.0132, respectively, which implied it was not significant relative to the pure error and indicated that the model equation was adequate for predicting the yield of brown pigment under any combination of values of the variables.

Table 3 Analysis of variance of the experimental result of the BBD

p-value Source	Sum of Squares	DF	Mean square	F-value	P-value
Model	0.031	9	3.466×10^{-3}	110.62	< 0.0001
X_1	2.665×10^{-3}	1	2.665×10^{-3}	85.05	< 0.0001
X_2	0.023	1	0.023	719.33	< 0.0001
X_3	1.170×10^{-4}	1	1.170×10^{-4}	3.74	0.0945
$X_1 X_2$	3.349×10^{-4}	1	3.349×10^{-4}	10.69	0.0137
$X_1 X_3$	7.396×10^{-5}	1	7.396×10^{-5}	2.36	0.1683
$X_2 X_3$	7.056×10^{-5}	1	7.056×10^{-5}	2.25	0.1771
X_1^2	4.861×10^{-4}	1	4.861×10^{-4}	15.52	0.0056
X_2^2	1.726×10^{-3}	1	1.726×10^{-3}	55.08	0.0001
X_3^2	2.683×10^{-3}	1	2.68×10^{-3}	85.65	< 0.0001
Residual	2.193×10^{-4}	7	3.133×10^{-5}		
Lack of Fit	2.006×10^{-4}	3	6.688×10^{-5}	14.34	0.0132
Pure Error	1.865×10^{-5}	4	4.663×10^{-6}		
Cor Total	0.031	16			

R^2_{adj} (adjusted determination coefficient) in Table 4 is the correlation measure for testing the goodness-of-fit of the regression equation. Higher it is the better degree of correlation between the observed and predicted values. The value of R^2_{adj} was 0.9840, which was reasonably close to 1 and implied that only less 0.2% of the total variations were not explained by model. Meanwhile, the predicted and observed coefficients of determination values for the above regression were close to each other, indicating that the model adequately fits the real relationship between the parameters chosen in this study. Coefficient of variation (CV) indicates the degree of precision with which the experiments are compared. A relatively low value of CV (0.9736) in Table 4, which showed a better precision and reliability of the experiments carried out.

Table 4: Analysis of variance for the fitted quadratic polynomial model of extraction

Item	Std. Dev.	Mean	C.V. %	R-Squared	Adj R-Squared	R-Pred Squared	Adeq Precision
Value	0.0056	0.5749	0.9736	0.9930	0.9840	0.8969	33.2295

3.2 Analysis of response surface

The 3D response surface and 2D contour plots are the graphical representations of regression equation. They provide a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables.

The interaction relationships between alcohol concentrations (X_1) and solvent: sample ratio (X_2) on the yield of brown pigment was shown in Fig. 1, which indicated that the yield of brown pigment enhanced rapidly with the increasing of X_2 , when the ultrasonic time was kept at 70 min. With the further increasing of alcohol concentrations X_1 , this trend became weak. As the Fig 1 shown, in spite of X_1 and X_2 exit some kind of interaction, it is not strong.

Fig. 2 shows that the contour of alcohol concentrations(X_1) and extraction time (X_3) present a circle like, which indicate the mutual interactions between the variables alcohol concentrations(X_1) and extraction time (X_3) are insignificant. Fig.3 has the similar situation like Fig.2. There is no obvious mutual interactions between the variables solvent: sample ratio (X_2) and extraction time (X_3).

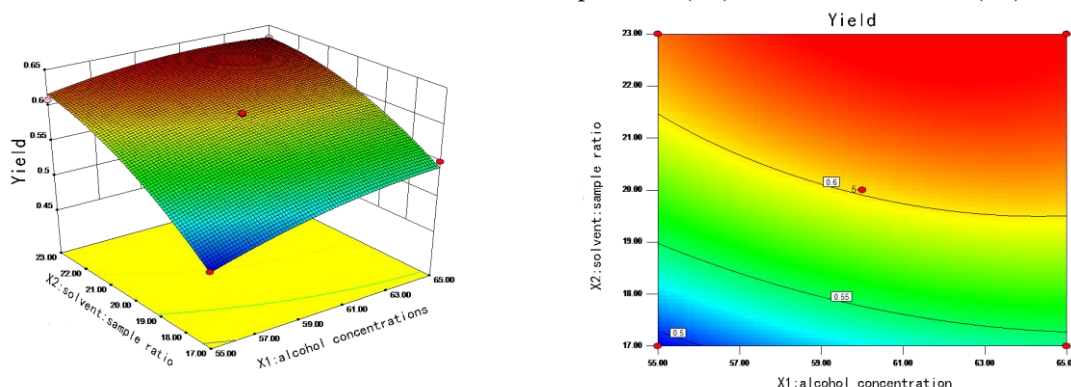


Fig.1 Response surface plot and contour plot of alcohol concentrations and solvent: sample ratio and their mutual interactions on the yield of brown pigment

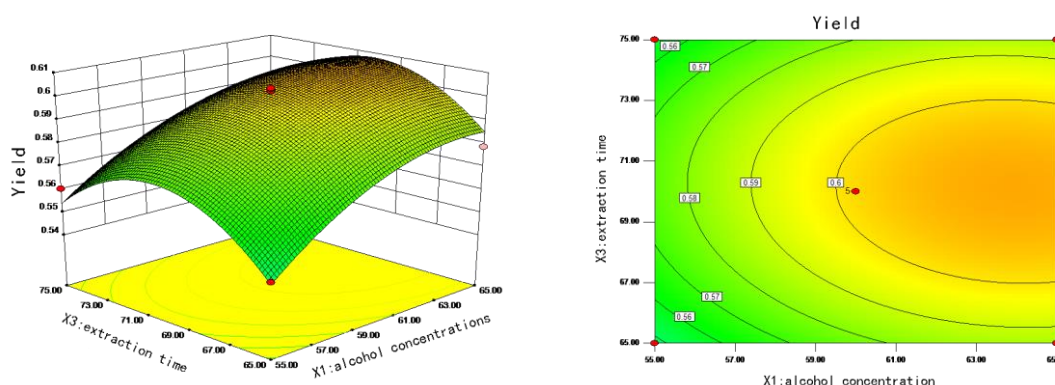


Fig.2 Response surface plot and contour plot of alcohol concentrations (X1) and extraction time (X3) and their mutual interactions on the yield of brown pigment

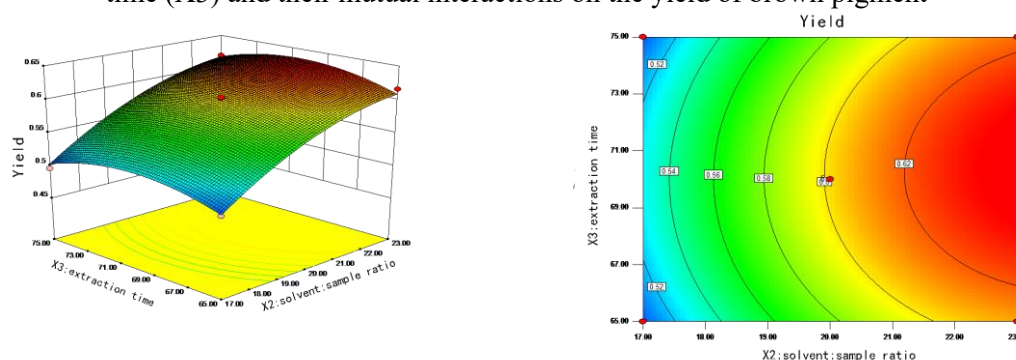


Fig.3 Response surface plot and contour plot of solvent: sample ratio (X2) and extraction time (X3) and their mutual interactions on the yield of brown pigment

3.3 Optimization of extraction parameters and validation of the model

The suitability of the model equation for predicting the optimum response values was tested using the selected optimal conditions. The maximum predicted yield was 0.6364g (Alcohol concentrations 61.99, Ratio of water to sample 23 mL/g, Ultrasonic time 70.63min). After modified the conditions, the experiment with maximum predicted yield was the 0.6360g (62, 23mL/g, 71min), which is in agreement with the predicted data.

4. Conclusion

The effects of UAE conditions on the quality of brown pigment from Macadamia shells were studied using RSM. It was significant that the effects of alcohol concentrations and solvent: sample ratio on brown pigment from Macadamia shells, as opposed to ultrasonic time. ANOVA showed a high coefficient of determination, $R^2 = 0.9930$, hence establishing a satisfactory adequacy of the models. The optimal extraction conditions for the brown pigment were determined as follows: ultrasonic treating time of 71min, solvent to sample ratio of 23mL/g, alcohol concentrations of 62. Under these conditions, the experimental yield of brown pigment was 0.636g, which is in good agreement with the predicted yield value.

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